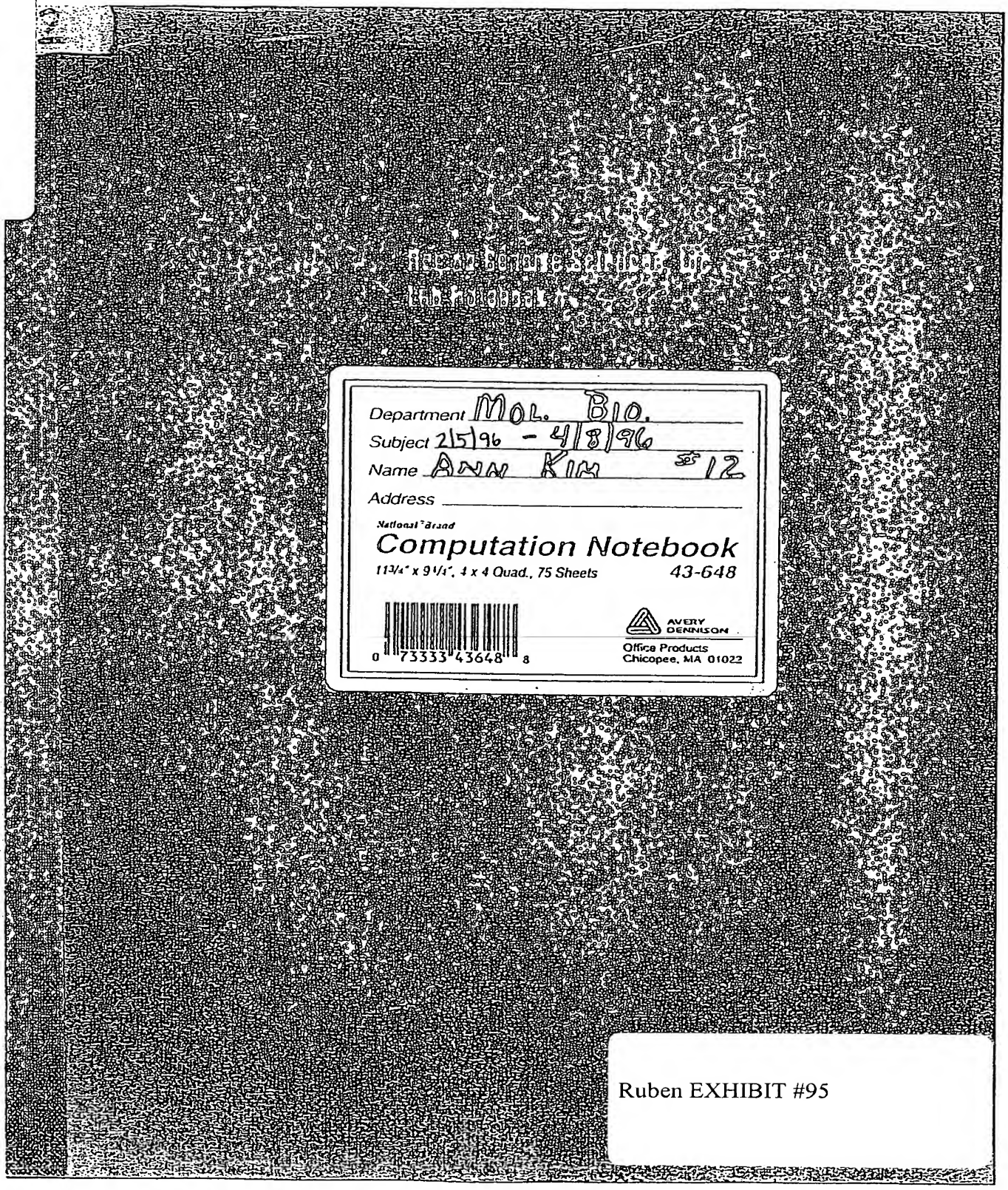
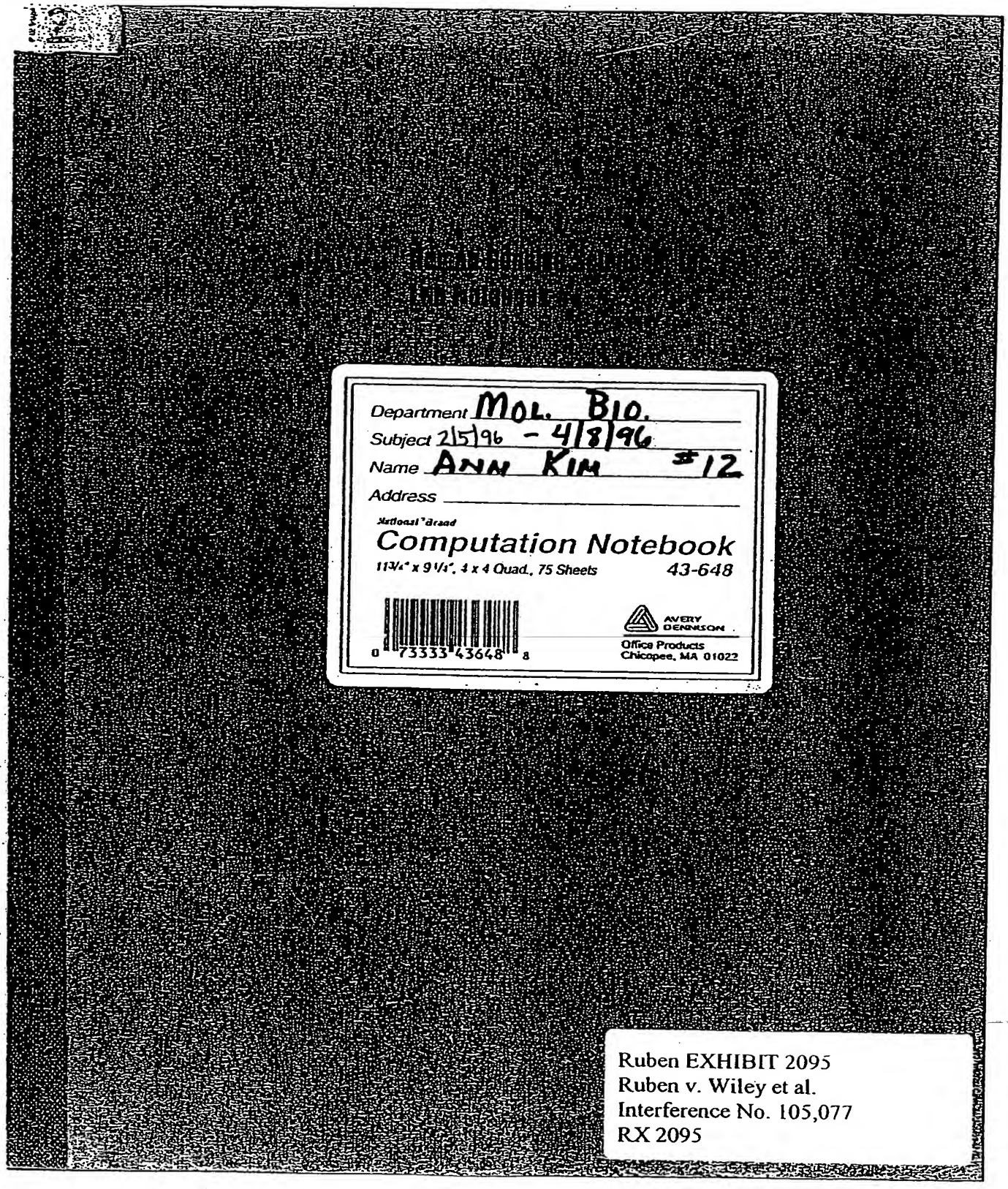


Ruben EXHIBIT #95

BEST AVAILABLE COPY



Ruben EXHIBIT 2095
Ruben v. Wiley et al.
Interference No. 105,077
RX 2095

2/6/96

DNA	4
10X#2	5
H_2O	40
EcoRI	0.5
XhoI	0.5
	50 µl.

Incubate 37°C O/N

GET Paperwork ready for

HTPAN08S04 +PQE6.
HSKBN09 +PQE7

HMSAF22 +GPPA2
HMSAF22 3'Δ +GPPA2
To Submit to Protein Expression.

Make Primers to Clone & into pCDNA 3' HA Tag

HE2PM21 - Soluble VEGF
HMSAF22 }
HT4SB02 } Possible secreted Protein.
HSKBN09 }

Make Primers to Clone into PQE6

HTPAN08S04-at New ATG Sight 1st for Cloning Core facility.

Inoculate for Maxi preps.

HTPAN08 +PQE6 } TB+Amp Kan
HSKBN09 +PQE7 }
HMSAF22 +GPPA2 } TB+Amp
HMSAF22B'Δ +GPPA2 }

2/7/96

Spin through G-25 Column - 1.3K.
Collect flow through
Count 6 ml

	SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#		RCH%
50ng	HSLED86	1	200	1	1258393.25	0.46	0.15	1.58	61.0	62.9×10^6	0.00
80ng	HSHBV83	2	201	1	1640159.88	0.40	0.15	3.38	112.0	82×10^6	0.00
50ng	HCVER32	3	202	1	1542533.25	0.42	0.15	5.16	87.0	77.15×10^6	0.00

Specific Activity cpm/ng

HSLED86 1.3×10^9 cpm/ng
HSHBV83 1.4×10^9 cpm/ng
HCVER32 1.5×10^9 cpm/ng.

Give Probes to Mark Porter for 2nd Blot

Prepare Mini Preps.

Spin Cultures 5K 15 min
Make Glycerol Stock of Cultures
Store -80°C.
Pour off Supernatant
Resuspend Pellet 10ml Buffer P1
Let sit at Room Temp 10 min
Add 10ml of Buffer P2
Add 10ml of ice cold Buffer P3
Incubate on ice 30 min
Spin 8K 30 min at 4°C
Equilibrate a Tip 500 with 10ml Buffer QBT
Apply Supernatant to Equilibrated Column through a Kim wipe
Allow Supernatant to Completely flow through Column Bed.

2/7/96

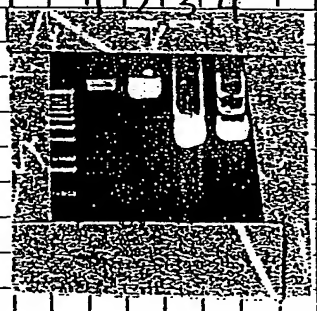
Wash column 2x with 30 ml Buffer QC
Elute in 15 ml Buffer QF
Add 0.7 Volumes (10.5 ml) of Isopropanol
Mix Well
Spin 8K 30 min
Pour off Supernatant
Wash pellet 15 ml 70% Ethanol
Spin 8K 15 min
Pour off Supernatant
Blot pellet to dry at Room Temp O/N

2/8/96

Qiagen Maxi S Continued
Resuspend pellets in 200 ul TE
Transfer to fresh tube
Read OD 260/280

Sample ID	abs 260.0 nm	abs 280.0 nm	260.0 nm / 280.0 nm	280.0 nm / 260.0 nm	
1 HMSAFZZ	0.0097	0.0040	2.4684	0.4055	0.1 ug/ul
2 HMSAFZZ3	0.0563	0.0360	1.5652	0.6389	0.56 ug/ul
3 HSKBN09	0.2331	0.1531	1.5226	0.6568	2.33 ug/ul
4 HTPAN08	0.0790	0.0507	1.5575	0.6421	0.79 ug/ul

Run 1ul on gel with 1 kb ladder



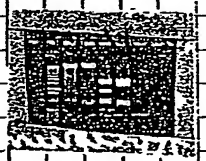
1) HMSAFZZ + GPPA2
2) HMSAFZZ 3Δ + GPPA2
3) HSKBN09 + PDE7
4) HTPAN08 + PDE6

2/8/96

Set up Digestions

	DNA	10X	H2O	Enzs.
1 HMSAFZZ+GPPAZ	10	2	7.6	0.2 BglII + AspHI
2 HMSAFZZ 3Δ+GPPAZ	1.8	2	15.8	0.2 BglII + AspHI
3 HSKBN09+PQE7	0.4	2	17.2	0.2 SapI + HindIII
4 HSPAN08H+PQE7	1.3	2	16.3	0.2 XhoI + HindIII

Incubate 37°C 4 hrs.
Run 10 µl on gel w/ 1Kb ladder
1, 2 + 4 look good + Correct
#3 ? more than one band ?
Incubate 37°C O/N.
Run again tomorrow



Set up reactions for PFU using PCR Optimization Buffers from Stratagene

10 mM Tris-HCl	$MgCl_2$	25 mM KCl	75 mM KCl
pH 8.3	1.5 mM	Opti-Prime™ 1x buffer #1	Opti-Prime™ 1x buffer #2
pH 8.3	3.5 mM	Opti-Prime™ 1x buffer #3	Opti-Prime™ 1x buffer #4
pH 8.8	1.5 mM	Opti-Prime™ 1x buffer #5	Opti-Prime™ 1x buffer #6
pH 8.8	3.5 mM	Opti-Prime™ 1x buffer #7	Opti-Prime™ 1x buffer #8
pH 9.2	1.5 mM	Opti-Prime™ 1x buffer #9	Opti-Prime™ 1x buffer #10
pH 9.2	3.5 mM	Opti-Prime™ 1x buffer #11	Opti-Prime™ 1x buffer #12

2/8/96

Use Different Sized DNA inserts

1	HTPB41S15	5' Bam	3' Xba
2	HTPAN08504	5' Nco	3' Xho
3	HE8CJ26	5' Bam	3' Asp
4	HT3AB35	5' Bam	3' HindIII

Use primers at - 20pmol / reaction
or 0.2 μg / reaction

DNA - at 100 ng/μl

Set up 13 reaction tubes per DNA sample

Add 5 μl of 10x Buffers 1-12
add 5 μl of PPU Buffer to #13

- Make Cocktail in the following Order.

* Make sure all reagents + Tubes are on ice *

	1	2	3	4
H_2O	41.2	270.6	231.2	469.6
50x Master Mix	12.5	12.5	12.5	12.5
10x dNTP	60	60	60	60
5' Primer	67.4	67.4	92.6	10
3' Primer	5.6	14.7	16.12	5.4
DNA	1	1	1	1
Taq	6.5	6.5	6.5	6.5
	565	565	565	565

45 μl / Reaction Tubes

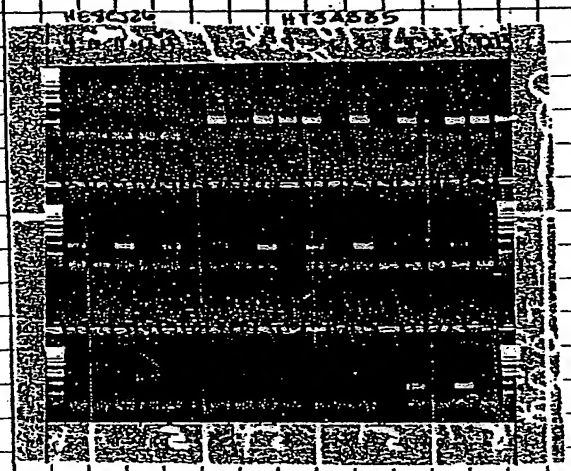
Aliquot into tubes first before ready to Start PCR

2/8/96

PCR PROG 71

95°C	5min
95°C	1min.
55°C	1min
72°C	1 min 45 sec.
72°C	7min
4°C	Hold.

Run 10 µl on gel w/ 1Kb ladder.



Looks like HTPB41515 was too weak to amplify — Need longer Extension time

For the most part it looks like the longer fragments like odd #s Buffers #1, 3, 5, 7.

TRY HTPB41515 Again using a longer Extension time!

2/9/96

Set-up Reactions for Core Cloning

Total of 18 samples —
16 mine
2 from John Green

2/9/96

			5' Primer	3' Primer
A	HUSAQ05	10ng/ul	4624	4623
B	HNFDW90	10ng/ul	4626	4625
C	HE8CJ26	250ng/ul	14602	14603
D	HT4SB02	250ng/ul	4636	4631
E	HMSAF22	250 ng/ul	4634	4638
F	H E2PM21	250 ng/ul	4633	4629
G	H SKBN09	250 ng/ul	4635	4630
H	HTPAN08	250 ng/ul	4632	14388

Set-up reactions using Buffers
\# 1, 3, 5, 7 & 9 - 10 ul/tube
Total of 200 ul of each Reaction

ON ICE

	A	B	C	D	E	F	G	H
H_2O	753	752	672	770.9	770.8	771.7	771	752.7
10x M.M.	20	20	20	20	20	20	20	20
10x dNTP	100	100	100	100	100	100	100	100
5 Primer	6	7	3.7	1.7	1.2	1.4	1.9	1.3
3 Primer	6	6	6.5	1.4	2	9.9	1.1	20
DNA	10	10	1	1	1	1	1	1
pfU (2.5u/ul)	5	5	5	5	5	5	5	5
	900 ul	900 ul	900 ul	900 ul	900 ul	900 ul	900 ul	900 ul

Aliquot 90 ul into each tube

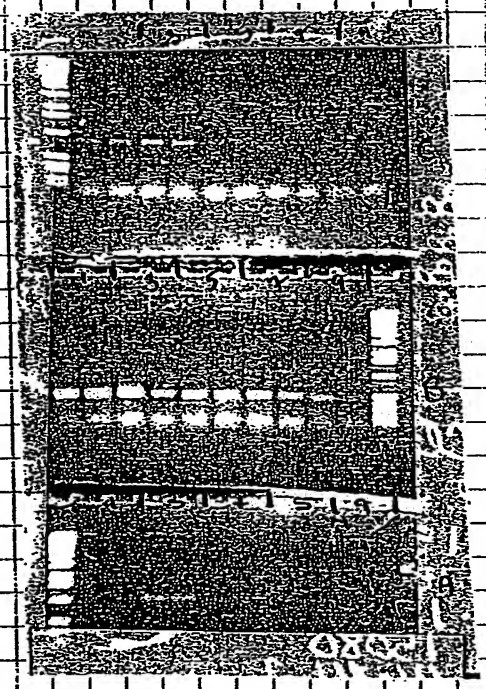
PCR

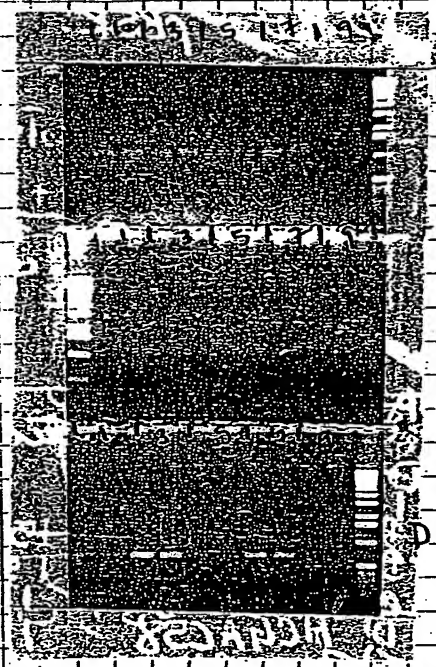
95°C 5 min
95°C 1 min
55°C 1 min
72°C 2 min
72°C 7.5 min
4°C Hold

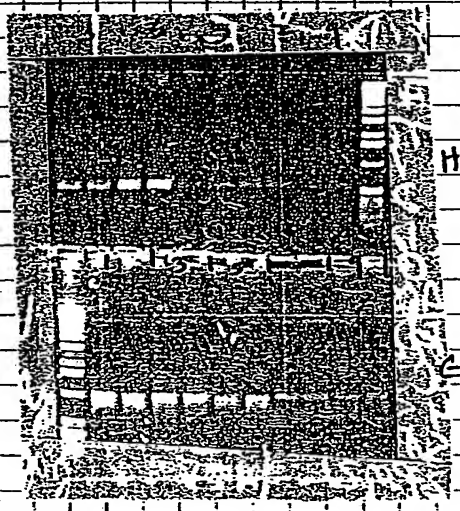
5 4629	0.3507	0.1921	1.8258	0.5477	2.31
6 4630	0.2808	0.1819	1.5434	0.6479	1.85
7 4631	0.2172	0.1518	1.4310	0.6988	1.43
8 4632	0.2431	0.1517	1.6032	0.6238	1.6
9 4633	0.2173	0.1450	1.4989	0.6672	1.43
10 4634	0.2653	0.1770	1.4992	0.6670	1.75
11 4635	0.1595	0.0989	1.6128	0.6201	1.05
12 4636	0.1778	0.1265	1.4057	0.7114	1.7

2/9/96.

Run Sul on gel w/ 1Kbladder







Combine Rxns that Worked into 1-2 tubes.

Pot w/ an Equal Volume 13% PEG 8000 / 1.0M NaCl.

Vortex well. Mix

Spin 15 min

Pour off Supernatant

Store -20°C till Monday

2/9/96

ROXANNE DUAN

Brought up samples for Cloning

Digest pBSK

DNA	3.7
10x Bam	5
H_2O	40.3
BamHI	1
	50

Incubate 37°C

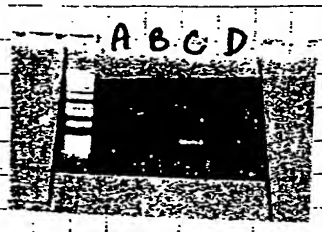
2/12/96

Set-up Reactions for Cloning

0.8Kb	A)	HSGSA[6]	15091	15077
0.3Kb	B)	HSGSA[6]	15091	15090
0.6Kb	C)	HCGAC58	15092	15089
0.16Kb	D)	HCGAC58	15092	15080
0.5Kb	E)	HAUCC34	4639	4641

	A	B	C	D	E
dH_2O	734.8	734.8	734.8	734.8	734.8
50X MM	20	20	20	20	20
10x dNTP	100	100	100	100	100
5' Primer	20	20	20	20	20
3' Primer	20	20	20	20	20
DNA	0.2	0.2	0.2	0.2	0.2
PFU	5	5	5	5	5
	900	900	900	900	900

2/12/96



Set-up Digests:

A	HSGSA61	15091 + 15077	Bam → PBSK
B	HSGSA61	15091 + 15090	Bam → PDO
C	HCGAC58	15092 + 15089	Bam → PDO
D	HCGAC58	15092 + 15080	Bam / Xho → pcDNA-3'HA

	A	B	C	D
DNA	20	20	20	20
10X	5	5	5	5
H_2O	24	24	24	23
Enz 1	1	1		1
Enz 2				1
	50	50	50	50

Incubate 37°C O/N.

Spin Samples from 2/9/96.
15 min
Pour off Supernatant
Wash Pellet 100 µl 70% Ethanol
Spin 5 min
Pour off Supernatant - let air Dry 15 min
Resuspend pellet in 50 µl TE

~~Set up Digests~~
Run 1 µl on gel w/ 1 Kb ladder

1	HUSAQ05	1.12
2	HUFLW90	0.48
3	HEGCJZ6	0.7
4	HT4SBD2	0.65
5	HMSAZZ3	0.92
6	HE2PM2J	1.1 Kb
7	HSLBN09	1 Kb
8	HTPONU6	0.57

Set up Digest pg 30

20

pg 16

2/12/96

A	HUSAQ05	Bgl II / Xho I	#2
B	HNFDW9D	BamHI / Xho I	Bam
C	HE8LJZ6	Bam I / Asp	B
D	HT4SBQZ	Bam / Xho	Bam
*E	HMSAFZZ	Bcl / Xho	M
F	HEZPMZ1	Bam / Xho	Bam
*G	HSKBNDT	Bcl / Xho	M
• H	HTPAN08	BspHI / HIII	B

	A	B	C	D	E	F	G	H
DNA	20	10	20	20	30	20	20	20
10X	5	5	5	5	5	5	5	5
H_2O	23	33	23	23	13	23	23	23
Enz 1	1	1	1	1	1	1	1	1
Enz 2	1	1	1	1	1	1	1	1

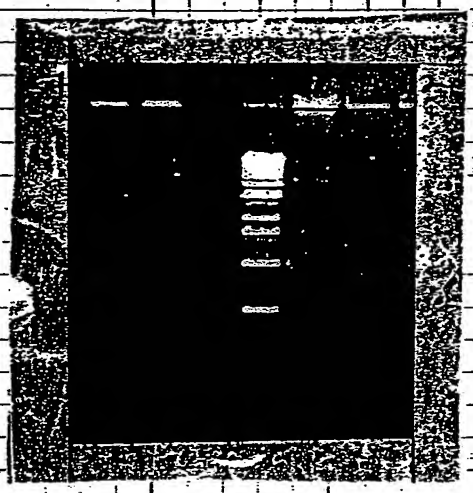
* For Bcl I Digests - Digest first in Xho I at 37°C - then Add Bcl and Incubate 50°C
• For BspHI - Need to use isoschizomer Rca I - No BspHI around

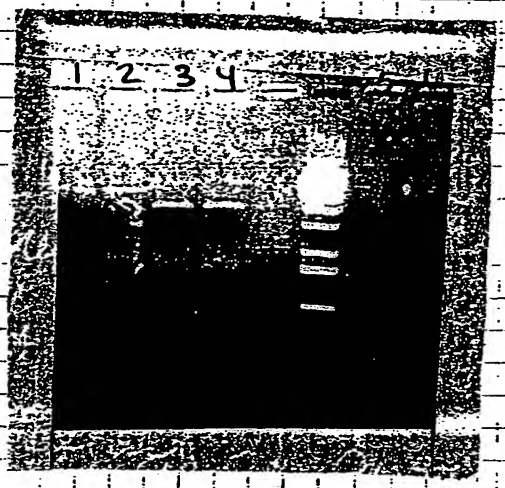
Incubate at 37°C 6 hrs

Aliquoted HTPAN08SOL 4B412
for Patient
25 Tubes w/ 100ng DNA at 10ng/µl
Give to Steve Ruben

2/13/96

Run Digests on LmP Gels
@ 80V 1½ hrs.
Cut out Gel Slice
Take Pictures





1	HCGAC58	Bam/Xho	pCDNA 3' HA
2	HCGAC58	Bam	pDIO
3	HSGSA61	Bam	pD10
4	HSGSA61	Bam	pBSK
5	HNFDW90	Bam/Xho	pCDNA 3' HA
6	HUSAQ05	Bgl II/Xho	pCDNA 3' HA
7	HEZPM01	Bam/Xho	pCDNA 3' HA
8	HE8BI26	Bam Xho	pA2
9	HMSAF22	Bcl I/Xho	pCDNA 3' HA
10	HSCBD09	Bcl I/Xho	pCDNA 3' HA
11	HT4SB07	Bam/Xho	pCDNA 3' HA
12	HTPAN08	BspH I/Hind III	pQE60

Gene Clean fragment

2/13/9(

Set up ligations

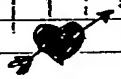
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1 HGGAC58 Bam/Xho	10	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
2 HCGAC58 Bam	X	10	X	X	X	X	X	X	X	X	X	X	X	X	X	X
3 HSGSA[e1 Bam	X	X	10	X	X	X	X	X	X	X	X	X	X	X	X	X
5 HNFDW90 Bam/Xho	X	X	X	10	X	X	X	X	X	X	X	X	X	X	X	X
6 HUSAQ05 Cla II/Xho	X	X	X	X	10	X	X	X	X	X	X	X	X	X	X	X
7 HEAM2/ Bam/Xho	X	X	X	X	X	10	X	X	X	X	X	X	X	X	X	X
8 HE8CJZ6 Bam/Nsp	X	X	X	X	X	X	10	X	X	X	X	X	X	X	X	X
9 HMSAF22 Bgl/Xho	X	X	X	X	X	X	X	10	X	X	X	X	X	X	X	X
10 HSKBN09 Bgl/Xho	X	X	X	X	X	X	X	X	10	X	X	X	X	X	X	X
11 HTUSB02 Bam/Xho	X	X	X	X	X	X	X	X	X	10	X	X	X	X	X	X
12 HTPA108 EcoRI/Pst	X	X	X	X	X	X	X	X	X	X	10	X	X	X	X	X
PCDNA 3'HA Bam/Xho	0.5	X	X	0.5	0.5	0.5	X	0.5	0.5	0.5	X	0.5	X	X	X	X
PD10 Bam	X	0.5	0.5	X	X	X	X	X	X	X	X	X	0.5	X	X	X
PA2 Bam/Nsp	X	X	X	X	X	X	0.5	X	X	X	X	X	X	0.5	X	X
PQE60 Nco/HIII	X	X	X	X	X	X	X	X	X	X	0.5	X	X	X	0.5	X
10X T4 Buffer	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
T4 Ligase	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
H_2O	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5	7
Total	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20

Incubate 16°C overnight

2/13/96

Inoculate LBT Amp with:
HTPAN08504
GPPA2 14
pCDNA 3HA

Incubate overnight 37°C w/aeration
overnight

2/14/96  VALENTINE'S DAY

Qiagen MAXI Prep.
HTPAN08504
GPPA2
PCDNA 3 HA

Spin 5K 15 min
Pour off Supernatant
Resuspend pellet 10 ml P1 Buffer
Add 10 ml P2 Buffer
Add 10 ml P3 Buffer
Incubate on ice 30 min.
Spin 8K 30 min.
Transfer Supernatant through
Kim wipe to Equilibrated
Tip 500.
Allow to flow through
Wash Tip 2x with 30 ml
QC Buffer
Elute with 15 ml Buffer QF
Collect - add 0.7 Volumes (10.5 ml)
of Isopropanol
Spin 8K 30 min
Pour off Supernatant
Wash pellet 15 ml 70% Ethanol
Spin 8K 15 min

2/14/96

Pour off Supernatant
Allow pellet to air Dry
Resuspend pellet in 200 µl TE
Read OD 260/280

Sample ID	abs 260.0 nm	abs 280.0 nm	260.0 nm / 280.0 nm	280.0 nm / 260.0 nm	
1	~~0.0388~~	~~0.0388~~			
2 HTPAN0850H	0.0457	0.0366	1.2477	0.8015	0.46 ug/ul
3 GIPPAL	0.1593	0.1044	1.5253	0.6556	1.59 ug/ul
4 PCDNA 3' HA	0.0458	0.0320	1.4240	0.7022	0.46 ug/ul

Set-up Digestions of the pCH. Prm 2/13.

PCH - 0.96 ug/ul.

PCDNA 3'H

	BamHI	XbaI	Asp 718	BamHI/XbaI	Bam/Xho
DNA	5.2 l	5.2 l	5.2 l	5.2 l	10.9
10X Buffer	5 Bam	5 #2	5 B	5 Bam	5
H_2O	37.79	37.79	37.79	36.79	30.1
Enzyme 1	1	1	1	1	1
Enzyme 2	—	—	—	1	1
	50	50	50	50	50

Incubate 37°C O/N

Transform ligations into chemically Competent Cells.
for pCDNA & PA2 - DH5α
pQE & pD10 - M15 up 5

2/14/96

Thaw Cells on ice
Aliquot 100 ul into pre-chilled tubes
Add 10 ul of ligation mix
Incubate on ice 1 hour
Heat 42°C 45 sec
Chill on ice
Add 400 ul LB
Incubate 37°C 1 hr
plate onto LB plates
pCDNA & PAZ - LB + Amp
PAE - PDIO - LB + Amp / Kan
Incubate 37°C overnight
Use ⊕ control PAZ 10 ng
& PAE 10 ng
Use ⊖ control of cells only.

2/15/96

Pick Colonies into LB + Amp or LB Amp / Kan in 96 well Deep
Incubate 37°C w/ aeration
4 hrs.
Set up PCR

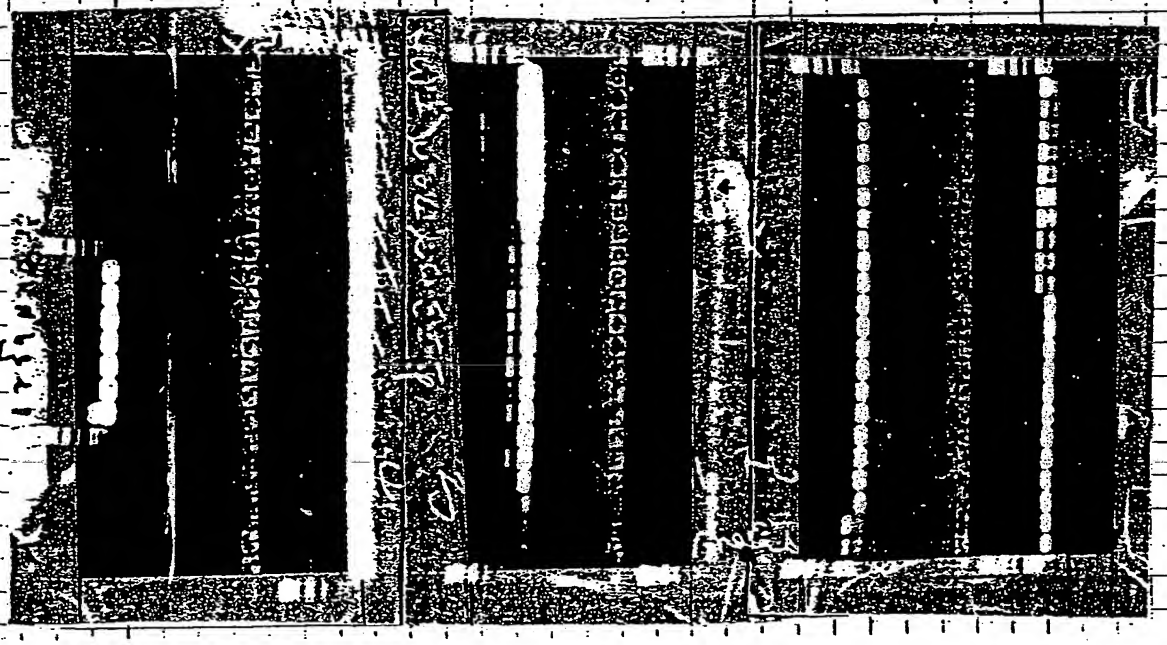
	A	B	C	D	E	F	G	H	I	J	K
10xPCR	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2
10xDNTP	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2
H_2O	21.4	22.3	22.3	20.4	21.4	20.3	21.4	19.4	20.4	21.4	23.2
Taq	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
cDNA	2	2	2	2	2	2	2	2	2	2	2
Primer #1	T7 1	PDIO 0.1	PDIO 0.1	T7 1	T7 1	PAE 0.1	T7 1	T7 1	T7 1	T7 1	PDIO 0.1
Primer #2	1	1	1	2	1	3	1	3	2	1	0.1

2/15/96

Primers

A	HCGAG58	pCDNA	#15000	10pmol/ul + T7
B	HCGAG58	pD10	#15000	10pmol/ul + PQE9/10
C	HSGSA61	pD10	#14810	10pmol/ul + PQE9/10
D	HNFDW90	pCDNA	#11693	7.7pmol/ul + T7
E	HUSAQ05	pCDNA	#14118	14.4pmol/ul + T7
F	HEPM21	pCDNA	FP02	3.2pmol/ul + T7
G	HESCJ26	PAZ	FP04 #H179	(10.5pmol/ul + T7 Bac
H	HMSAF22	pCDNA	FP07 11579	C3 2pmol/ul) + T7
I	HTSCW09	pCDNA	FP06 11463	(5.6pmol/ul) + T7
J	HTYSB02	pCDNA	FP05 11746	9.0 pmol/ul + T7
K	HTPMN08	pQE6	FP14	+ PQE 6/6

PCR Program Cole.
Run 10 ul on gel w/ 1kb ladder



2/15/96

Inoculate for Mini Preps

Try to PCR inserts with Taq

Take some of PCR fragment Generated from 2 Pg 12/12/96

PCR Using Same primers. + 0.4 ul Taq. — Total of 500 µl Rxns.

PCR.

95°C	5 min	
95°C	1 min	25x
55°C	1 min	
72°C	1:45 min	
72°C	7:30 min	

Run 5 ul on gel w/ 1 Kb ladder

1 HCGAC58
2 HCGAC58
3 HSGSA61
4 HSGSA61
5 HNFDW90
6 HUSAQ05
7 HEPM21
8 HESC126
9 HMSAF22
10 HSKBN09
11 HTGSP02
12 HTPAN08

PEG PPT.

Wash pellet 1000 µl of 70% Ethanol

Spin 5 min

Pour off supernatant

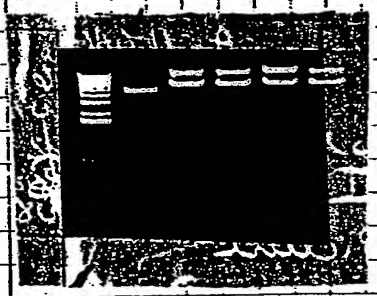
2/15/96

Allow pellet to Air Dry
Resuspend in 20 (or) 40 µl TE
Set-up Digests

	1	2	3	4	5	6	7	8	9	10	11	12
DNA	25	25	25	25	25	25	25	25	25	25	25	25
10X Buffer	5	5	5	5	5	5	5	5	5	5	5	5
H_2O	18	19	19	19	18	18	18	18	18	18	18	18
Enz 1	1 Xho	1 Bam	1 Bam	1 Bam	1 Bam	1 BglII	1 Bam	1 Bam	1 BCl	1 BCl	1 Bam	1 Bam
Enz 2	1 Bam				1 Xho	1 Xho	1 Asp	1 Xho	1 Xho	1 Xho	1 Xho	HCII
	50	50	50	50	50	50	50	50	50	50	50	50

Incubate 37°C O/N.
With BCl Digests - incubate at 50°C for 4 hrs before Running on Low PGel

Run 2ul of PC4 Digests ng plus 1 Kbladder



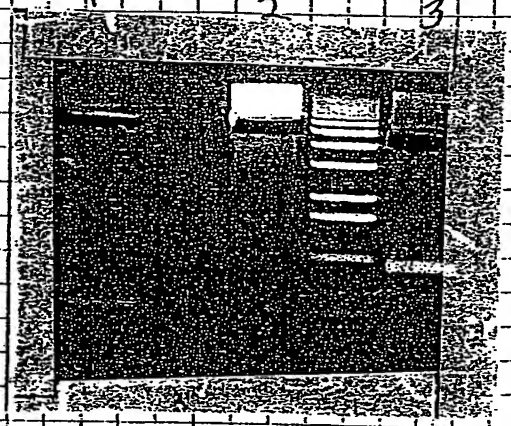
1 pCDNA 3HA Bam/XhoI
2 Asp 718
3 Bam #1
4 Xba
5 Bam/Xba
} pC4

Run 1/2 of #1 & 5 on LMP Gel with PC1 Bam/Xba and 1 Kbladder

Run at 80V 2 hrs
Cut out DNA Fragment
Take Picture

30

2/15/96



1 PCI Bam/Xba

2 PCH Bam/Xba

3 pCDNA 3-HA Bam/Xho

Store -20°C O/N

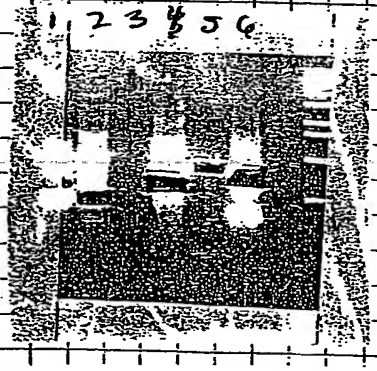
Sample ID	abs 260.0 nm	abs 280.0 nm		260.0 nm / 280.0 nm	280.0 nm / 260.0 nm	
1 PCI 5'(-)	0.1281	0.0779	24 mer	1.6454	0.6078	0.85 µg/µl 106.8 pmol/µl
2 MCS rppi 3'(-)	0.1530	0.1037	21 mer	1.4745	0.6782	1 µg/µl 145.7 pmol/µl

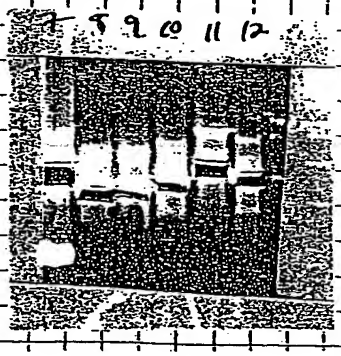
2/16/96

Run Digests on 0.8% LMP Gel w/ 1K bladelder.

Run 80V 2hrs

Cut out gel Slices & take Picture





2/Ke/96

Melt Gel slices at 68°C
Set up ligations

	A	B	C	D	E	F	G	H	I	J	K	L
2 HCGACSB B	10											
4 HSGGA61 B		10										
5 HNFDW90 B/K			10									
6 HNGAQ05 B/K				10								
7 HCPM21 B/K					10							
8 HEGC26 B/K						10						
9 HMSAFU B/K							10					
10 HSEBN09 B/K								10				
11 HTYSB04 B/K									10			
12 HTPAX8 B/HIII										10		
10X Liga Buf.	5	5	5	5	5	5	5	5	5	5	5	
H_2O	33.5	33.5	33.5	33.5	33.5	33.5	33.5	33.5	33.5	33.5	H40	
T4 Ligase	1	1	1	1	1	1	1	1	1	1	1	
pcDNA 3 HA B/K			0.5	0.5	0.5		0.5	0.5	0.5			
pD10 Bam	0.5											
pBSK Bam		0.5										
pA2 Bam Asp						0.5						
pQE6 N/HIII										0.5		
	50	50	50	50	50	50	50	50	50	50	50	

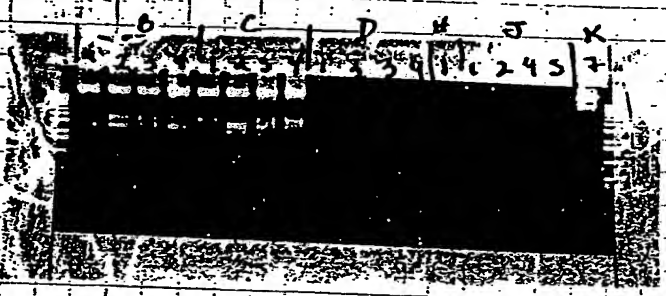
Incubate at Room Temp 2 hrs.
Store 4°C
Company Closed 1pm due to Weather.

Baling Minipreps.
Spin 10 ml Cultures
Remove Supernatant
Resuspend pellet in 700µl STET + RNase.
Heat to 100°C 5 min

2/16/96

Spin 15min
Transfer 650 µl of Supernatant to
Add Fresh Tube
Vortex. 650 µl of 13% PEG / 1.6M NaCl
Spin 15min
Pour off Supernatant
Wash Pellet 1000 µl 70% Ethanol
Spin 5min
Remove Supernatant
Allow Pellet to Air Dry 15 min
Resuspend in 100 µl TE.

Run 1 µl on gel vs 1 Kb ladder



B HCGAG5 X-PD10
C HSSA6 - PD10
D HNFDW10 - pC 3' HA
H HMSAF22 - pC 3' HA
J HTYSB02 - pC 3' HA
K HTPAN88 pBEC

Set up digestion. B/C - with Bam and K w/ Hind III ~~Bam K no I~~

None Digested.
Redo Next Son Monday

2/19/96 OEF Presidents Day

2/19/96

Transform ligations from 2/16/96

Transform PQE & PDIO constructs into M15 up4

Transform PAZ, PBSK + PcDNA 3'HA into DH5α cells.

Thaw Chemically Competent cells on ice
Aliquot 100ul into Sterile Tubes
Add 10µl of Ligation Reaction
Incubate on ice 1 hr
Heat 42°C for 45 sec
Quick chill on ice
Add 400ul LB
Incubat 37°C 1 hr.
plate onto LB + Antibiotics plates.
M15 App up4 cells — LB + Amp/Kan
DH5α cells — LB + Amp.
Incubate 37°C O/N

Inoculate for Mini preps. - 2/16/96

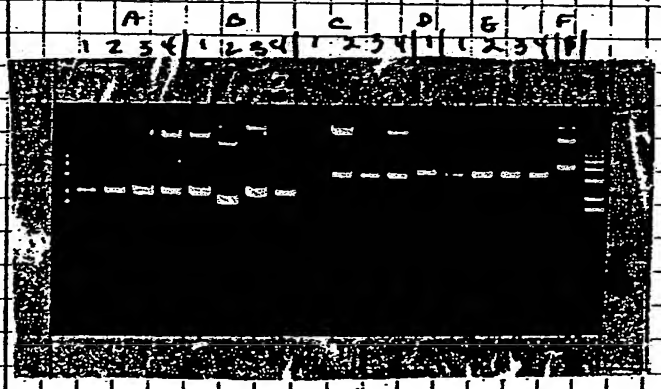
2/20/96

plate are again contaminated
— just LB + Amp —
picked clones anyway
into 96 well Dish
Incubate 37°C O/N

Mini preps —
Do Promega preps

2/20/96

Spin 2 ml culture 2 min
Remove Supernatant
Resuspend pellet in 500 µl Resuspension Buffer
Add 500 µl Cell Lysis Buffer
Mix by inverting tube several times
Add 500 µl of Neutralization Solution – mix well –
Spin 15 min
Transfer Supernatant to fresh tube (with 1000 µl of Resin Slurry –
Incubate 5 min at RT
– Use Column with 3cc syringe attached
Apply Resin & Supernatant to Column
Vacuum through
Wash 2 x 1ml Wash Buffer
Spin Column 1 min
Apply 150 µl TE – Heated to 68°C
Let Sit 5 min
Spin 1 min & Collect
Run 1µl on gel w/ 1 Kb ladder



A – HCGAG58 + PDO
B – HSGSA61 + PDO
C – HNFDW90 + PC3'
D – HMSAF22 + PC3'
E – HTG5802 + PC5'
F – HTPAN08 + PDE6

2/24/96

PCR Cultures

A - HCGAC98 Bam + PD10 Bam
C - HNFDW90 Bam/Xho + pcDNA 3'HA
D - HUSAQ05 BglII/Xho + pcDNA 3'HA
E - HE2PM21 Bam/Xho + pcDNA 3'HA
F - HTGBCJ26 Bam/Asp + pA2
G - HMSAF22 Bcl/Xho + pcDNA 3'HA
H - HSKBN09 Bcl/Xho + pcDNA 3'HA
I - HTYSB02 Bam/Xho + pcDNA 3'HA
J - HTPAN08 BspH/HIII + pQE60

C, D, E, G, H, I - pcDNA 3'HA

		72X
T7	1	72
Sp6	2	144
10x dNTP	3.2	230.4
10x PCR	3.2	230.4
H_2O	20.4	1468.8
Cult	2	
Taq	0.2	14.4
	30ml	20ml/Tube

F - pA2

		12X
T7 Bcl	0.05	0.6
3' Asp 718	3	36
10x dNTP	3.2	38.4
10x PCR	3.2	38.4
H_2O	20.4	244.8
cult.	2	
Taq	0.2	2.4
	32	30ml/Cult

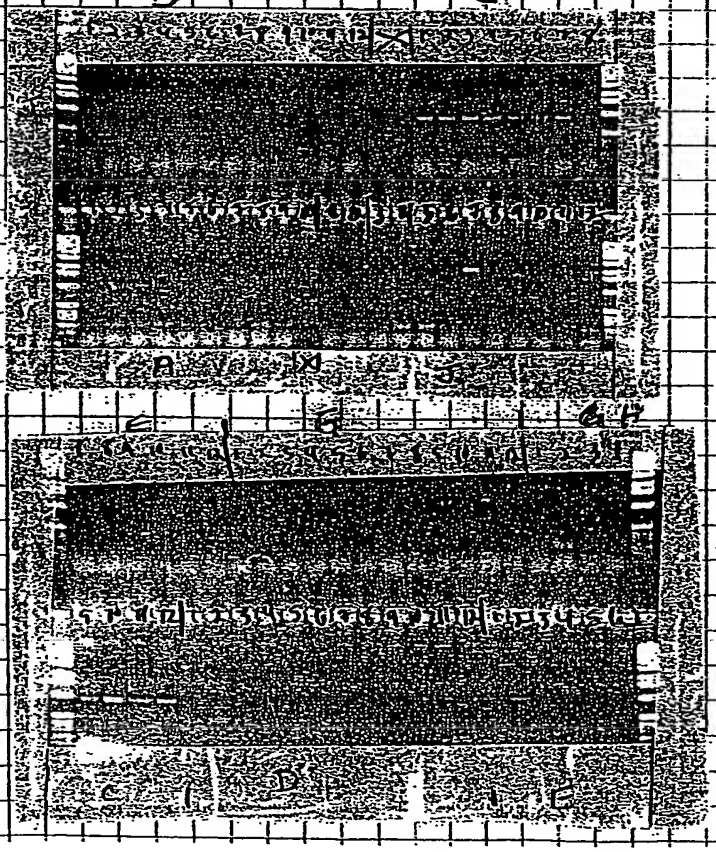
2/21/96

AJ - PD10 + POE60.

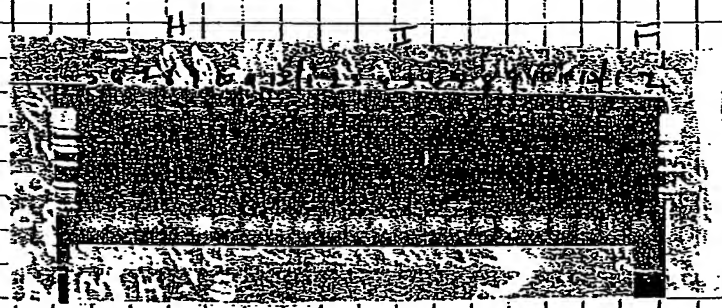
		33X
3' POE	0.2	6.6
7' POE	1	33
10X	3.2	105.6
10X	3.2	105.6
H2O	22.2	732.6
Cult	2	—
Tag	0.2	6.6
	30ml	30ml/well

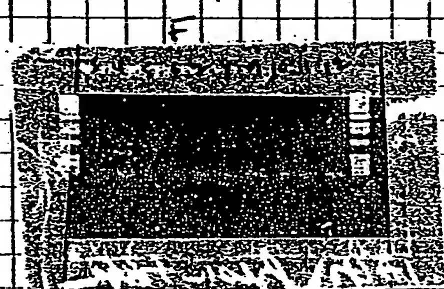
PCR Program 66.

Run 10ul on gel w/ 1Kb ladder



2/21/96





- Inoculate some pBSK - HSGSA(?) Bam into 5ml TB + Amp.
- Inoculate:
  HNEDW90 C1-4
  HUSAQ05 D1-D5.
  HERPMD21 E2.

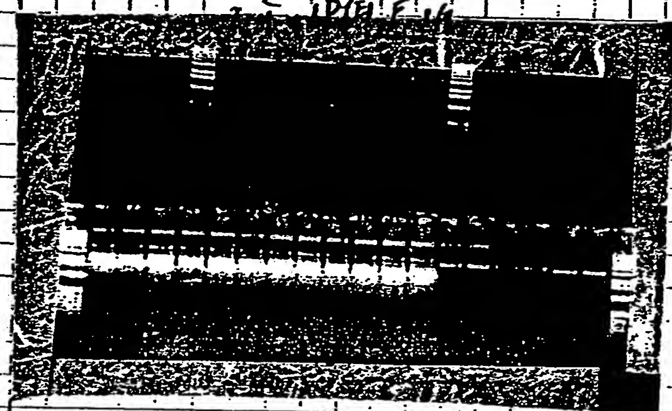
  into TB+Amp. for Boil mini preps

2/22/96

Promega Mini prep DNA
see pg 33-34. for Protocol.

2/22/96. Setup

Run 1μl on gel with 1Kb ladder



A - HSGSAG1 + PBSK
B - HNFDW90
C - HUSAR05
D - HE2EM24
E - HMSAF22
F - HSKBN09
G - H7PAN08

Set-up Digests

pcDNA 3-HA
HIII / Xba

		(22x)
DNA	10	
10X #2	3	66
100X BSA	0.3	6.6
H2O	16.3	358.6
HIII	0.2	4.4
Xba1	0.2	4.4
	30	30μl/tube

pBSK
XbaI

		(13X)
DNA	4	
10X #2	3	39
BSA (100x)	0.3	3.9
H2O	22.5	292.5
XbaI	0.2	2.6
	30	30μl/tube

pD10 & pDE60.
EcoRI / HIII

		12X
DNA	10	
10X #2	3	36
100X BSA	0.3	3.6
H2O	16.3	195.6
HIII	0.2	3.6
EcoRI	0.2	3.6
	30μl	30μl/Tube

Incubate 37°C
4hrs.

2/27/96

Set up Digests
-EcoRI / & Xho I.
Dilute DNA to 250 ng/ul

		5x0
DNA	8	
10 x EcoRI	5	25
H_2O	36	180
EcoRI	0.5	2.5
Xho I	0.5	2.5
	50	42ul / tube

also Digest HCE3026 1.37 ug/ul
HCE5E84 1.57 ug/ul

DNA	4
10 X EcoRI	5
H_2O	40
EcoRI	0.5
Xho I	0.5
	50.0

Incubate 37°C O/N.

Carrie + I Packed up lab #15 on 3rd floor 9020. Will be moving to Lab #5 2nd floor 9020.

2/28/96

Carrie + I spent the whole day moving Boxes & Moving Stuff to New Space.

2/29/96

Set-up PCR of colonies picked 2/28/96

1. HMSAF22 + GPPAZ — T7 + 3' BglII
2. HMSAF22 3'Δ + GPPAZ — T7 Bac + 3'ΔAsp.
3. HCEED20 + GPPAZ — 13627 # [illegible] T7 Bac
4. HCEGG495 + pCDNA 3' HA
5. HFGAm58 + pCDNA 3' HA
6. HATCK 81 + pCDNA 3' HA

} cloe 3' + 5'

	1x	6x
T7 Bac	0.1	0.6
3' BglII	2	12
10x dNTP	3.2	19.2
10x PCR	3.2	19.2
H_2O	21.2	127.2
Taq	0.3	1.8
Cell	2	
	32 µl	30 µl/tube

	1x	6x
T7 Bac	0.1	1.2
3Δ Asp.	3	36
10x dNTP	3.2	38.4
10 x PCR	3.2	38.4
H_2O	20.2	242.4
Taq	0.3	3.6
Cell.	2	
	32.0	30 µl/tube

	1x	25x
T7	0.1	2.5
3' 13627	0.4	10
10x dNTP	3.2	80
10x PCR	3.2	80
H_2O	21.8	545
Taq	0.3	7.5
Cell	2	
	32 µl	30 µl/tube

	1x	75x
3' Cho	0.02	1.5
5' Cho	0.02	1.5
10x dNTP	3.2	270
10x PCR	3.2	270
H_2O	23.26	1744.5
Taq	0.3	22.5
Cell.	2	
	32 µl	30 µl/tube

PCR Prog C06

95°C	5 min	
95°C	30 sec	30×
55°C	30 sec	
72°C	1 min	
72°C	10 min	
4°C	Hold	

2/28/96

3/5/96

Count Gel.

SAM	POS	CH	CPM	2SIGX	TIME	EL TIME	AVG H#
HCE3U2L4	1	296	1	728784.00	0.47	0.25	1.72	60.0
HMEGE64	2	297	1	815745.00	0.50	0.20	3.48	61.0
HCESF89	3	298	1	890950.00	0.47	0.20	5.26	65.0

7.3 X 10^5 /ml
8.2 X 10^5 /ml
8.9 X 10^5 /ml

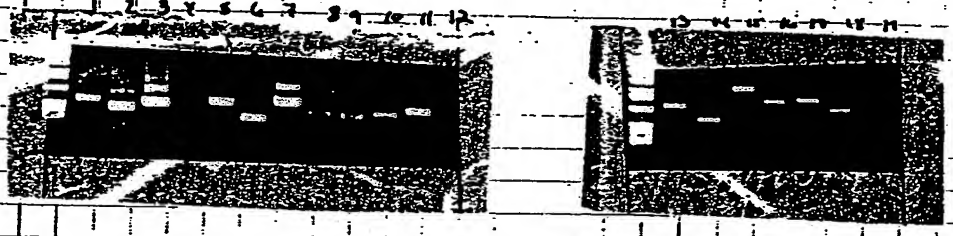
Give Prob + DNA Strips to Mark Porter.

Phenol Chloroform Extract Gel slices
from 2/16/96 - pg. 30
from 2/29/96 - pg 62.

Add TE to 500 µl
Heat 68°C
Add 500 µl heated phenol (1)
Mix well
Spin 10 min
Transfer Supernatant to fresh tube
Add 500 µl phenol
Mix well
Spin 10 min
Transfer Supernatant to fresh tube
Add 800 µl SEVAG
Mix well
Spin 10 min
Transfer Supernatant to fresh tube
Add 40 µl 3M NaAct. pH 5.3
Add 1000 µl 100% Ethanol
Mix well
Let Sit on ice 10 min
Spin 15 min

3/5/96

Pour off Supernatant
Wash Pellet 100µl 70% Ethanol
Spin 5 min
Pour off Supernatant
Allow Pellet to Air Dry 15 min
Resuspend pellets in 40µl TE
Run 1% agarose w/ 1 KB ladder



1	HCGAC58	Bam/Xho	
2	HCGAC58	Bam	
3	HCGAC58	HSGSAC61	Bam/H1
4	HNFDW90	Bam/Xho1	
5	HUSAQ05	BglII/Xho1	~800bp
6	HUSAQ05	BglII/Xho1	~450bp
7	HE2PM21	Bam/Xho1	
8	HE8CJ26	Bam/Asp	
9	HMSAT23	Bcl/Xho1	
10	HSKBN07	Bcl/Xho1	
11	HTYSB02	Bam/Xho1	
12	HTPAD08	Bgl/H/HTF	
13	HLTB050		Eco RI/Xho1
14	HCE3026	0.7kb	Eco/Xho1
15	HCE3026	2.5kb	Eco/Xho1
16	HPRDH07		Eco/Xho1
17	HFTDJ62		Eco/Xho1
18	HPRCA03		Eco/Xho1
19	HTYEM77		Eco/Xho1

3/6/96

Set up ligations of ones that have not worked in the past

	1	2	3	4	5	6	7	8	9	10	11	12
HCEED20 A+P	4											
HSGSA61 Bam		4										
HCGAC58 Bam			4									
HUSAQ BglII/Xho				4								
HSUAQ BglII/Xho					4							
HTRANS8 BspHI/HindIII						4						
HCGAC58 Bam/Xho							4					
pBSK Kpn	0.5							0.5				
pBSK Bam		0.5	0.5						0.5			
pCDNA 3'HA Bam/Xho				0.5	0.5		0.5			0.5		
PQE6 NcoI/HindIII						0.5					0.5	
10X Buffer	2	2	2	2	2	2	2	2	2	2	2	2
H_2O	12.5	12.5	12.5	12.5	12.5	12.5	12.5	16.5	16.5	16.5	16.5	17
T4 Ligase	1	1	1	1	1	1	1	1	1	1	1	1
	20	20	20	20	21	20	20	20	20	20	20	22

2A2

28

After ligate @ at RT O/N

- Therapeutic Protein Meeting 12:15 - Carrie Fischer Presentation on TNTS

3/7/96

Gathered Plasmid preps of all Constructs with HG Project Code

3/7/96

HTPAN08 - HG03500 - Fab Legend
pBL HG03500 - pBSK
pE9 HG03500 - pD10 Septum
AZ HG03500 - PAZ Construct
pHA HG03500 - pCDNA3 HA Tag

HTPB911 - HG06300 - Villin
pBL HG06300
pE9 HG06300
pE60 HG06300 - pQE60
AZ HG06300

HCDAA59 - HG09900 - HEZPM21
pBL HG09900
AZ HG09900

HSKBN09 - HG10900
pBC HG10900
pE7 HG10900 - pQE7
AZ HG10900

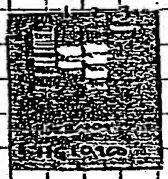
HT4SB02 - HG09700
pBC HG09700
AZ HG09700
pHA HG09700

HMSA002 - HG03700 - HMSAF2
pBC HG03700

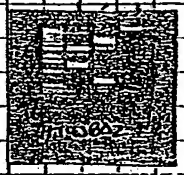
HNBAA26 - HG09800
pBC HG09800

HNFAA64 - HG10000
pBL HG10000

3/7/96.



1- HSKBN07 Xho/EcoRI
2- HSKBN09 + pQE7 - SphI/HindIII
3- HSKBN09 / PA2 - Bam/Xba



1- HTUSB07 Xho/EcoRI
2- HTUSB02 + pCDNA 3 HA Bam/Xho
3- HTUSB02 + PA2 Bam/Xba

1- HMSAF22 Xho/EcoRI

1- HMPSA79 - Xho/EcoRI
2- HMPSA79 + pQE60 Bam/BglII

Transform ligations (pg 75) from 3/6/96.

for pQE6 constructs use M15 up4 cells
or for pBSK/pCDNA 3' HA constructs use XL-1 Blue cells.

3/7/96.

Use Chemically Competent frozen Cells.

- Thaw cells on ice
- Aliquot 100 ul into first sterile tube
Add 10 ul of Ligation
Incubate on ice 1 hour
Heat 42°C for 45 sec
Quick Chill on ice
Add 400 ul of LB
Incubate 37°C 1 hour
plate 100 ul on two plates.
pBSK - LB + Amp + IPTG / Xgal
pCDNA 3' HA - LB + Amp.
pQE6 - LB + Amp / Kan

Incubate at 37°C O/N.

3/8/96.

Inoculate 200 ul of LB + Amp
with (96 well Dish)
HUSAQ05 + pCDNA 3' HA Tag (60)
HCGAC58 + pBSK - (36)
HCEED20 + pBSK - (24)
HSGSA61 + pBSK (36)

Inoculate 200 ul of LB + Amp / Kan
with (96 well Dish)
HTPAN08 + PQE6 96.

Incubate all at 37°C w/ aeration.

Set up PCRs

12/3/96

HUSAQ05. + PCDNA 3 HA

		62x
T7 PCI	0.3	18.6
1414	1	62
10x dNTP	3.2	198.4
10x PCR	3.2	198.4
H_2O	22	1386
Taq	0.3	18.6
Cult	2	
	32µl	30µl/tube

HTRAN08 + PQE60.

		100x
T5 PQE	0.02	2
3' PQE	0.25	25
10x dNTP	3.2	320
10x PCR	3.2	320
H_2O	23.13	2313
Taq	0.2	20
Cult.	2	
	32µl	30µl/tube

HSQSA61 + PBSK.

		38x
M13R	0.1	3.8
4810 (10pmol)	1	38
10x dNTP	3.2	121.6
10x PCR.	3.2	121.6
H_2O	22.3	847.7
Taq	0.2	7.6
Cult.	2	
	32µl	30µl/tube

HCGAC58 + PCDNA 3 HA

		38x
T7	0.3	11.4
PC 3'	0.1	3.8
10x dNTP	3.2	121.6
10x PCR	3.2	121.6
H_2O	22.9	870.2
Taq	0.3	11.4
Cult	2	
	32µl	30µl/tube

HCEED20 + PBSK

		26x
M13R/F	0.1	2.6
10x dNTP	3.2	83.2
10x PCR	3.2	83.2
H_2O	23.3	605.8
Taq	0.2	5.2
Cult.	2	
	32µl	30µl/tube

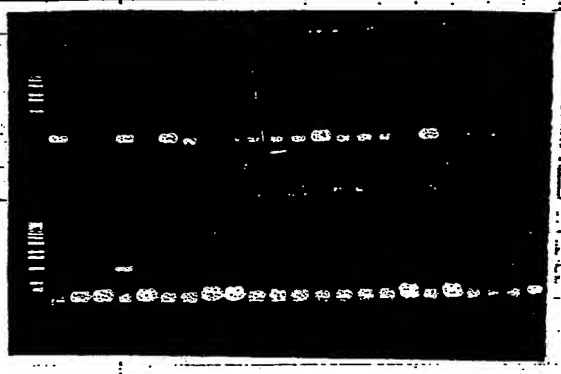
PCR.

	95°C	5min.
30x	95°C	30sec.
	55°C	30sec.
	72°C	1min.
	72°C	7 min.
	4°C	hold.

3/8/96

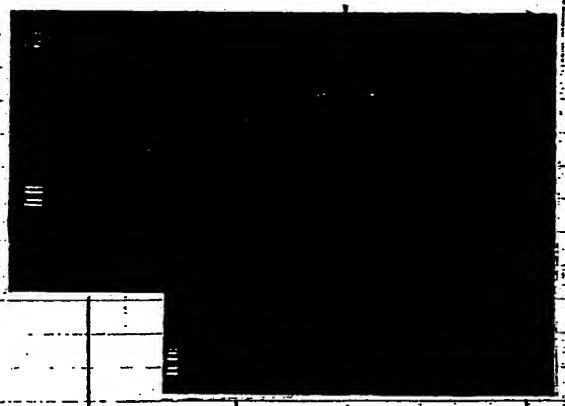
Run 10 uL of PCR Rxn w/ 1Kb ladder

HTPAN08 + PQE6



HTPAN08 + PQE6.

HUSAQ05 + pCDNA 3' HA.



3/15/96

Inoculate TBS Amp for mini prep's on Monday leave at RT till monday.

Qiagen mini prep.

HE2BM21 +3 HA
HMSAF22 +3 HA
HSKBW09 +3 HA
HTUSB02 +3 HA
HTPANO8 + PQE6.

PPT + Allow to air dry till monday.

Set up ligations

		1	2	3	4	5	6	7
HTCAF20 BINCO		5						
HTHCV08	Bam/Asp	—	5	—				
HCEED20	Asp	—	—	5	—			
PQE60 NCO	Bam	1	—	—	1	—		
PCR	Bam/Asp	—	1	—	—	1	—	
PBSK	Asp	—	—	1	—	—	1	—
10x Buffer		2	2	2	2	2	2	2
T4 Ligase		1	1	1	1	1	1	1
H2O		11	11	11	10	10	10	17

Incubate at RT overweekend

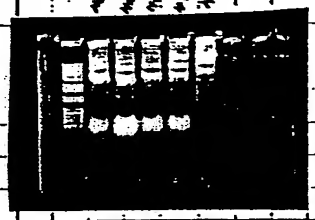
Split TC Cells.

3/18/96

Qiagen Maxi Cont.

Resuspend pQE6 construct 400 µl TE

Resuspend 3'HA Construct in 200 µl TE
Run 1 µl on gel w/ 1 kb ladder.



1 kb of RNA in 3'HA constructs
Qs to 500 µl –
Add 500 µl PEG/NaCl
Mix well
Spin 15m
Wash pellet 1000 µl 70% Ethanol
Spin 5m
Remove Supernatant
Allow pellet to air Dry 15m
Resuspend in 100 µl TE
Read OD 260 – 280 1:200 Dilution

Sample ID	abs 260.0 nm	abs 280.0 nm	bkg abs 320.0 nm	260.0 nm / 280.0 nm	260.0 nm / 280.0 nm
1 HE2Pm21 + 3'HA	0.1206	0.0775	0.0005	1.5594	0.6413
2 HMSAF221 3'HA	0.0934	0.0606	0.0036	1.5765	0.6343
3 HSKBN01 + 3'HA	0.0706	0.0457	0.0044	1.6017	0.6243
4 HT4SB02 + 3'HA	0.1050	0.0701	0.0063	1.5474	0.6463
5 HTCAN03 + pQE6	0.1173	0.0771	0.0073	1.5751	0.6349

Clark Kent Ultra
Set up Digestions

HT4SB02 } 2 Bam/Hind
HE2Pm21 }

HSKBN01 } 2 Kpn/Hind
HMSAF22 }

HTCAN03 + pQE6 } 2 Eco/Hind III

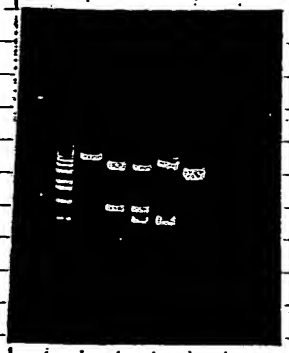
3/18/96

DNA	2
10X	3
BSA	0.3
Bam	0.2
Xho	0.2
H_2O	24.3
	30µl

DNA	2
10X	3
BSA	0.3
Kpn	0.2
Xho	0.2
H_2O	24.3
	30µl

DNA	2
10X	3
EcoRI	0.2
HindIII	0.2
H_2O	24.6
	30µl

Incubate 37°C 4hrs
Rxn 10 µl on gel with 1 kb ladder



HE2PM21 is linearized
HMSAF22 - looks correct w/ RNA contamination
HSKBN09 - internal site?
HT45B02 - incomplete digestion
HTPAN08 - incomplete digestion
Digest for longer & Re Rxn.

Inoculate TB+Amp or Amp Kan with Cultures

HSGSA61 Bam+PBSK = A1, A2, B3, B4, D5, D6.
HCGAC58 Bam+PBSK = E10, E9, F9, F11, F12, G1, G2, H3, H4.
HTPAN08 (Nco/HIII) + PQE 60 = A4, C4, D2
HELBS34 + PC4 = D10
HELBS34 + PBSK = G5, G6, G8
HCEED20 + GPPA2 = A1, A2, B3, B4, C5, C6, D7, D8.
Incubate 37°C O/N.

3/18/96

Transform Ligations into
Chemically Competent cells
XL-1 cells

Thaw cells on ice
Aliquot 100µl of cells into fresh tubes
Add 10 µl of ligation
Incubate on ice 1hr
Heat 42°C 1 min
Chill on ice
Add 2400µl LB
Incubate 37°C 1 hr
Plate onto 2 LB+Amp plates for
PCR + LB+Amp Beta-Gal for
pBSR.
Incubate 37°C O/N

Freeze Cole Cells.

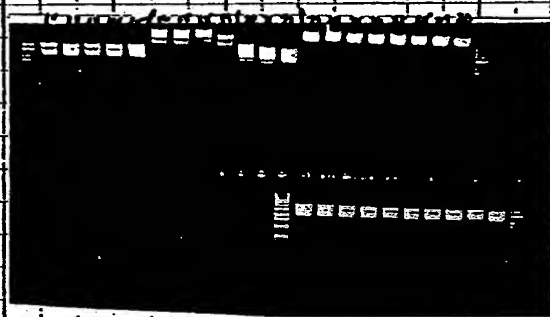
3/19/96

Alkaline Lysis Mini Prep

Spin 2ml culture 2 min.
Remove Supernatant
Resuspend pellet 300µl Resuspension
Buffer
Add 600µl Cell Lysis Buffer
Add 300µl Neutralization Buffer
Mix well
Let sit on ice 15 min
Spin 15 min
Transfer Supernatant to fresh tube
Add equal Volume 13% PEG/
1.6M NaCl
Mix well
Spin 15 min
Remove Supernatant

3/19/96

Wash pellet 1000μl 70% Ethanol.
Spin 5min
Remove Supernatant
Allow pellet to air dry 15 min
Resuspend pellets in 180μl T.E.
Run 2μl on gel with
1Kb ladder



1-6 HSGSAG1 + PBSK

7-15 HCGAC58 + PBSK

16-18 HTPAN08 + PQE6.

19-22 HELB5+Ry + PBSK

23-30 - HCEED20 + GPPA2

Mini Preps look good.
Set-up Digestions

HSGSAG1 + PBSK
HCGAC58 + PBSK
PBSK

HTPAN08 + PQE6
PQE60.

		16x
DNA	10	—
10X Bam	3	48
BSA	0.3	4.8
Bam	0.2	3.2
H_2O	16.5	264
	30μl	20μl/tube

		4x
DNA	10	—
10X	3.0	12
BSA	0.3	1.2
Nco	0.2	0.8
Hind III	0.2	0.8
H_2O	16.3	65.2
	30μl	20μl/tube

3/19/96

Set up Digests of
1 HMSAF22 + GPPA2 (BglII/Asp)
2 HMSAF22 3'D + GPPA2 (BglII/Asp)

	①	②
DNA	22	10
10X	5	5
H_2O	21	33
BglII	1	1
Asp	1	1
	50µl	50µl

Incubate 37°C
4 hrs

Ran 1µl of Digest on gel with 1Kb ladder

looks like correct digestion

Ran on LMP Tomorrow.

Make Probes. HTPAN08, HTXEM77, HSHBI83
H-FTDJG62

	SAM	POS	CH	CPM	2SIG%	TIME
HTPAN08	1	15	1	550513.31	0.49	0.30
HFTDJG62	2	16	1	945610.00	0.46	0.20
HTXEM77	3	17	1	761804.00	0.46	0.25
HSHBI83	4	18	1	813240.00	0.50	0.20

northerns (HTPAN08, HFTDJG62, HTXEM77)
screen HTD lib
screen filters from CLF

3/20/96

Re Ran Digests from 3/18 (pg 104)

3/20/96.

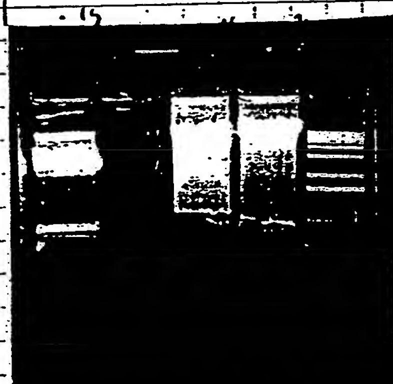
1 HEZPM21 - Bam/Xho
2 HMSAF22 - Kpn/Xho
3 HSKBN07 - Kpn/Xho
4 HT45B02 - Bam/Xho.
5 HTPAN08 - Nco/Hh

pcDNA has 2 Kpn Sugars to give ~886bp fragment

HT45B02 looks correct.
HSKBN07 looks correct

Need more Digests of HEZPM21 HTPAN08
HMSAF22
~~HT45B02~~

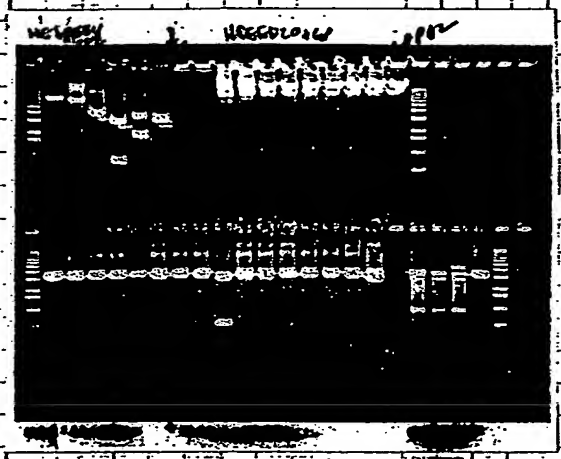
Run HMSAF22 on LMP Gel with 1 Kb ladder.
loaded Samples after Carrie Started Run
Cut out fragment
Take picture



Gel slice ready for clean up or ligation

3/20/96

Rsm Digests of Miniprep's.



Bam Digests look like they linearized but did not "Pop out" fragment except HEGHC58. #3. looks correct.

HTPAN08 + PQEG None look correct. No insert?

HECB532. Should Give ~400bp fragment That was Digested w ~600bp ASK Peter.

HCEED20 – Hard to tell Runs 0.8% or Low gel

Wash. HTO filters.
0.2xSSC
0.1% SDS.
65 C 1 hr. } 3X. on film

– Lab meeting

3/22/96

CORE NORTHERN BLOT SUBMISSION FORM

Gene Name: [redacted]

[redacted]

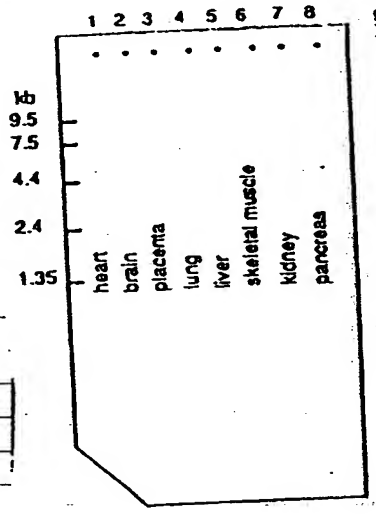
[redacted]/ug):

[redacted] x 10[redacted]

3 X

[redacted]een obser

[redacted]ith the above information, filled out the probe [redacted]
[redacted]bridization membrane with a sample of [redacted]
(DNA is [redacted] the probe diluted over a 5 log range (1 ng, 100 pg, 10 pg, 1 pg, 0.1 pg). Boil the DNA dilutions for 5', Spot the DNA on the membrane, using no more than 5 ul per spot. Alternately, denature the samples in Southern denaturation solution. In either case, crosslink the strip.



gram of your Northern ba
sity of your band. We w

[redacted]rea

[redacted] $S^{32}S$), $I_{32}(S^{32}S)$

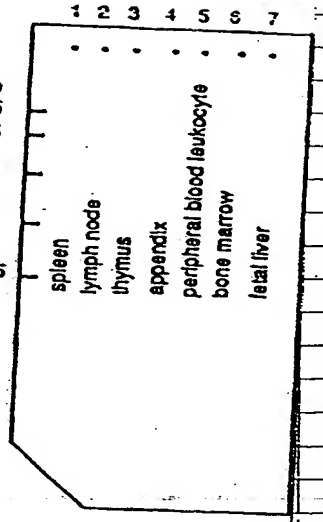
[redacted]ol

[redacted]0.3×10^5

Ext. SHOWED NOTHING.

[redacted]: 17-50), 15', 42°C

2x, .2×SSC/.1% SDS, 30' EACH, 65°C



TNT Results: 4/2/96

TNT RESULTS 4/2/96

INVESTIGATOR	SAMPLE NAME	EXPECTED SIZE (KDa)	OBSERVED SIZE (KDa)
REINHARD EBNER	HOECW07	51.8	FAINT BANDS AT 23,30,33
YAJUN CHEN	HPMDH16 FRAME 1	48	48 (?) also a background band at 48 but band in your sample is more intense
YAJUN CHEN	HPMDH16 FRAME 2	48	48 (?) also a background band at 48 but band in your sample is more intense
YAJUN CHEN	HPMDH16 FRAME 3	48	48 (?) also a background band at 48 but band in your sample is more intense
ANN KIM	HTPAN08 + PCDNA	30	30
ANN KIM	HTPAN08 + 3'HA	30	30, 35
ANN KIM	HT4SB02 + PCDNA	24	NO PRODUCT OBSERVED
ANN KIM	HT4SB02 + 3'HA	24	NO PRODUCT OBSERVED
ANN KIM	HSBAW14 + GPPA2	26	24
CHARLES FLORENCE	HBMSE33	10	NO PRODUCT OBSERVED
T7 POSITIVE CONTROL	DNASE 02-105	33	OFF GEL - WILL REPEAT SAMPLE
T7 NEGATIVE CONTROL	NO DNA	NONE	33
T3 POSITIVE CONTROL	HCAC183	33	NONE
T3 NEGATIVE CONTROL	NO DNA	NONE	33
REACTIONS PERFORMED BY: CARRIE FISCHER			NONE

Make Probes for Northerns.

HTPAN08
HTRACB12
HLTB050
~~HSHB088~~

SAM	POS	CH	CPM	2SIG%	TIME
1	296	1	545253.31	0.49	0.30
2	297	1	930665.00	0.49	0.20
3	298	1	883680.00	0.48	0.20

4/4/96

CORE NORTHERN BLOT SUBMISSION FORM

Gene Name: Fas Ligand

Clone Name (e.g. HS A186) HTPAN08

cDNA size (in kb [redacted]

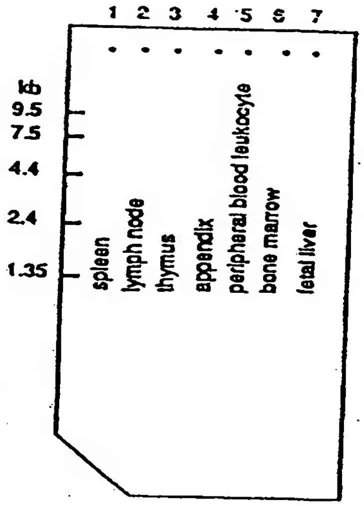
Specific Activity [redacted]

(MINIMUM ACC[redacted] m/ug)

Total counts [redacted]

Libraries in whic[redacted]

Other [redacted]



You must give us [redacted] above infor and a control strip of nylon hybridization membr. DNA used to make the probe diluted over a 5 log r pg, 0.1 pg). Boil the DNA dilutions for 5'. Spot th using no more than 5 ul per spot. Alternately, de Southern denaturation solution. In either case, c

You will receive the autoradiogram of your Nort... 3 to 4 days, depending on intensity of your band. We will mark the film with the sizes of the marker ladder.

for Dave 4/5/96

QC information

Name of hybridizer: MARK PORTER

Date hybridized: 4-3-96

Blots hybridized Im, #1

Hybridization Solution: Hybrisol

Counts/ml hyb. buffer added: 32.7 ×10^5

Exposure time: 1-DAY

Wash conditions: .2xSSC/.2% SDS

1x, 15', 42°C

2x, 20', 65°C

PPt w/ Ethanol + 3M Na Acetate
spin lukanged
Read OD 260/280

Sample ID	abs 260.0 nm	abs 280.0 nm	bkg abs 320.0 nm	260.0 nm / 280.0 nm
1 HMSAF23 HGPA2	0.1731	0.1134	-0.0008	1.5[illegible]
2 HMSAF23 HGPA0	0.1488	0.0968	-0.0026	1.5240

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